Understanding biological data via MD: the hIFNγ glycosylation puzzle

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Abstract

Human interferon-gamma (hIFNγ) is a key immunomodulating secretory glycoprotein. The biologically active form of the cytokine represents a non- covalent homodimer, each of the monomers being 143 aa residues long. The natural cytokine is a glycoprotein with two N-glycosylation sites in each monomer chain – Asn25 and Asn97, which are independently and differentially glycosylated. Although glycosylation is not necessary for its activity, it does affect the physico-chemical properties of the cytokine. There is experimental evidence that glycosylation promotes the folding and dimerization of the recombinant protein and also protects hIFNγ from proteolytic degradation, thus extending its circulatory half-life.

Recently, it was found that when labeled with His6-FLAG tag added to their N-termini, both glycosylated (expressed in insect cells) and non- glycosylated (expressed in E. Coli) forms of the fusion protein exhibit 100 times lower that expected biological activity. Upon removal of His6-FLAG tag the non-glycosylated forms recover their activity, while in the glycoforms the tag becomes enterokinase-resistant and cannot be removed.

We present a MD study on human hIFNγ to shed light on the mechanism through which glycosylation preserves the integrity of the cytokine molecule. We report the development of several in silico models of glycosylated native hIFNγ fusion proteins. By means of long-scale MD simulations we reveal the restrictive role of the glycans for the wagging motion of the highly volatile C-termini by keeping them within a reduced ensemble of conforma- tions closer to the globular part of the cytokine. That way, the functionally important sequences in the globule are preserved from protease attacks in the blood flow. At the same time, sporadic individual contacts between the gly- cans and the C-termini allow the proteolysis of the last several amino acids that yields more stable and biologically active forms of the cytokine with different truncation grades. Thus, we are faced with a double-sided mechanism of glycosylation-governed activity modulation by decreasing the entropy of the initial state (responsible for the stabilisation of the hINFγ molecule and prolongation of its circulatory half-life, at typical activity levels) and by increasing the enthalpy of the final state (related to the appearance of differently truncated forms, some of them with a higher biological activity). Ultimately, both are favorable for the biological function of the cytokine, so glycosylation is indeed the key to the stability and the preserved high activity of hIFNγ.

The above findings are presented in two recent papers:

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